

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Previously presented) A method of identifying RNA ligands which bind to a target molecule, said method comprising:

preparing, through one or more rounds of amplification and selection, a first pool of RNA ligands that collectively bind more than one target, wherein the first pool of RNA ligands comprises one or more predominate target-binding RNA ligands and one or more non-predominate target-binding RNA ligands;

treating the first pool of RNA ligands under conditions effective to reduce the concentration or eliminate the presence of the one or more predominate target-binding RNA ligands from the first pool of RNA ligands;

amplifying the RNA ligands in the treated first pool, thereby forming a second pool of RNA ligands that is enriched in one or more non-predominate target-binding RNA ligands of the first pool but not the one or more predominate target-binding RNA ligands thereof; and

identifying from the second pool one or more predominate target-binding RNA ligands that are present in the second pool at a higher concentration than other target-binding RNA ligands.

2. (Original) The method according to claim 1 further comprising:

treating the second pool under conditions effective to reduce the concentration or eliminate the presence of one or more predominate target-binding RNA ligands;

amplifying the RNA ligands in the treated second pool, thereby forming a third pool of RNA ligands that is enriched in one or more non-predominate target-binding RNA ligands of the second pool but not the one or more predominate target-binding RNA ligands thereof; and

identifying from the third pool one or more predominate target-binding RNA ligands that are present in the third pool at a higher concentration than other target-binding RNA ligands.

3. (Original) The method according to claim 2 wherein each said treating comprises:

introducing into the pool to be treated one or more nucleic acid molecules that hybridize to the one or more predominate target-binding RNA ligands to form hybrid complexes and

introducing into the pool to be treated an enzyme which cleaves at least the RNA ligand of the hybrid complexes, thereby destroying the one or more predominate target-binding RNA ligands.

4. (Original) The method according to claim 1 further comprising repeating said treating, amplifying, and identifying for each subsequent pool until substantially all of the non-predominate target-binding RNA ligands in the first pool have been identified.

5. (Original) The method according to claim 4 wherein each said treating comprises:

introducing into the pool to be treated one or more nucleic acid molecules that hybridize to the one or more predominate target-binding RNA ligands to form hybrid complexes and

introducing into the pool to be treated an enzyme which cleaves at least the RNA ligand of the hybrid complexes, thereby destroying the one or more predominate target-binding RNA ligands.

6. (Original) The method according to claim 5 wherein the one or more nucleic acid molecules are DNA and the enzyme is an RNaseH enzyme.

7. (Original) The method according to claim 4 wherein each said identifying comprises:

isolating the one or more predominate target-binding RNA ligands and sequencing the one or more predominate target-binding RNA ligands.

8. (Original) The method according to claim 1 wherein said identifying comprises:

isolating the one or more predominate target-binding RNA ligands and sequencing the one or more predominate target-binding RNA ligands.

9. (Original) The method according to claim 1 further comprising: preparing the pool of RNA ligands that collectively bind to more than one target and

identifying one or more predominate target-binding RNA ligands.

10. (Original) The method according to claim 9 wherein said preparing comprises:

expressing a library of RNA molecules that includes both RNA ligands that bind to at least one of one or more targets and RNA molecules that do not bind any of the one or more targets; and

partitioning the library of RNA molecules to form the first pool of RNA ligands.

11. (Original) The method according to claim 10 wherein said expressing the library of RNA molecules comprises:

expressing a library of DNA molecules that includes both DNA ligands that bind to at least one of one or more targets and DNA molecules that do not bind any of the one or more targets; and

transcribing the library of RNA molecules from the library of DNA molecules.

12. (Original) The method according to claim 1 wherein said treating comprises:

introducing into the first pool one or more nucleic acid molecules that hybridize to the one or more predominate target-binding RNA ligands to form hybrid complexes and

introducing into the first pool an enzyme which cleaves at least the RNA ligand of the hybrid complexes, thereby destroying the one or more predominate target-binding RNA ligands.

13. (Original) The method according to claim 12 wherein the one or more nucleic acid molecules are DNA and the enzyme is an RNaseH enzyme.

14. (Original) The method according to claim 1 wherein the targets comprise natural or synthetic small molecules, macromolecules, supramolecular assemblies, and combinations thereof.

15. (Previously presented) A method of reducing the concentration or eliminating the presence of unwanted target-binding species from a pool of RNA ligands, said method comprising:

providing a pool of RNA ligands which includes both wanted and unwanted target-binding RNA ligands;

isolating one or more unwanted target-binding RNA ligands;

sequencing the one or more unwanted target-binding RNA ligands;
introducing into the pool one or more nucleic acid molecules that hybridize to
the one or more unwanted target-binding RNA ligands to form hybrid complexes; and
introducing into the pool an enzyme which cleaves at least the RNA ligands of
the hybrid complexes, thereby destroying the one or more unwanted target-binding RNA
ligands.

16-17 (Cancelled)

18. (Previously presented) The method according to claim 15 wherein the
one or more nucleic acid molecules are DNA and the enzyme is an RNaseH enzyme.

19. (Previously presented) The method according to claim 15 wherein the
one or more unwanted target-binding RNA ligands comprise one or more RNA ligands that
bind to a matrix used to partition the pool of RNA ligands from a library of RNA molecules.

20. (Original) The method according to claim 15 wherein the unwanted
target-binding RNA ligands are RNA ligands that bind to a matrix, and wherein said treating
comprises:

introducing into the pool one or more nucleic acid molecules that hybridize to
the RNA ligands that bind to a matrix, thereby forming hybrid complexes and

introducing into the pool an enzyme which cleaves at least the RNA ligands of
the hybrid complexes, thereby destroying the RNA ligands that bind to a matrix.

21. (Original) The method according to claim 20 wherein the one or more
nucleic acid molecules are DNA and the enzyme is an RNaseH enzyme.

22. (Original) The method according to claim 20 wherein the matrix is a
nitrocellulose matrix.

23-46 (Cancelled)

47. (New) A method of identifying RNA ligands which bind to a target
molecule, said method comprising:

preparing, through one or more rounds of amplification and selection, a first
pool of RNA ligands that collectively bind more than one target, wherein the first pool of
RNA ligands comprises one or more predominate target-binding RNA ligands and one or
more non-predominate target-binding RNA ligands;

treating the first pool of RNA ligands under conditions effective to reduce the concentration or eliminate the presence of the one or more predominate target-binding RNA ligands, but not the one or more non-predominate target-binding ligands, from the first pool of RNA ligands;

amplifying the RNA ligands in the treated first pool, thereby forming a second pool of RNA ligands that is enriched in one or more non-predominate target-binding RNA ligands of the first pool but not the one or more predominate target-binding RNA ligands thereof; and

identifying from the second pool one or more predominate target-binding RNA ligands that are present in the second pool at a higher concentration than other target-binding RNA ligands.